THE MECHANISM OF THE SHADOW REFLEX IN CIRRIPEDIA. II. PHOTORECEPTOR CELL RESPONSE, SECOND-ORDER RESPONSES, AND MOTOR CELL OUTPUT[1]

G. F. GWILLIAM

Department of Biology, Reed College, Portland, Oregon 97202, and the Marine Biological Laboratory, Woods Hole, Mass. 02543

In a previous report (Gwilliam, 1963), certain electrical events at various locations in the nervous system of cirripedes, associated with changes in the light level impinging upon a photoreceptor, were described. At that time it was suggested that the photoreceptor cells have axons that do not synapse until reaching the supraesophageal ganglion, and that these cells influenced the activity of the second-order neurons by passive electrotonic conduction of a depolarizing potential which occurs when the photoreceptor is illuminated. Recent evidence from electron microscopy (Fahrenbach, 1965) supports this suggestion from a structural point of view, and observations to be reported here lend functional support.

Recent papers on the structure of the crustacean nauplius eye (Kauri, 1962; Elofsson, 1963) indicate that it is made up of three components, and the evidence presented here that adult balanid barnacles possess three (paired lateral and single median) photoreceptors suggests that they may well be derived from the three-parted naupliar eye found in the larvae of *Balanus* (Kauri, 1962). While it appears that the detailed structure of the three "compartments" of the larval medial eye does not coincide with that of the presumed separated components of the adult photoreceptors, the mere existence of three distinct components indicates a possible developmental source of the three adult photoreceptors.

In addition, further information has been obtained on neural pathways from the ocelli to certain of the muscles responsible for the withdrawal-closure response to a shadow that is so characteristic of most barnacles.

Materials and Methods

Three species of barnacles have been used in this study. *Balanus eburneus* Gould was used for the intracellular recording, and the animals were supplied by the Supply Department of the Marine Biological Laboratory, Woods Hole, Mass. Other observations were made on specimens of *Balanus tintinnabulum* (L.) and *Balanus cariosus* (Pallas). The former were supplied by Dr. Eric Barham, Navy Electronics Laboratory, San Diego, California, and Dr. James Case, University of California, Santa Barbara. The latter were collected by the author from the north central Oregon coast.

The lateral eyes of *B. eburneus* were exposed by splitting the shell along the longitudinal (rostrocarinal) axis, carefully removing the opercular valves with the

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body of the animal attached, leaving two "half-shells," each of which bears a lateral eye and a short length of retinula cell axons. The eye is located at the junction of the fused rostrum-rostrolateral and lateral shell plates and is easily visible with the naked eye. These "half-shells" were then mounted, inner surface up, with soft wax, in the recording chamber. The pigmented mantle over the photoreceptor was then dissected away, and the capsule of the tapetum or reflecting layer was removed. This permitted direct viewing of the photoreceptor cells, which appeared as two orange-yellow areas in each eye, although histological examination reveals three cells.

Next, the preparation was treated with trypsin (× 300), 80 mg./100 ml. of sea water, for 45 minutes. Following this treatment, penetration with the micropipettes could be accomplished in many preparations. Attempts to penetrate cells without enzymatic pre-treatment were never successful.

Glass micropipettes filled with 3 *M* KCl, having a resistance of 10–15 megohms in sea water, served as electrodes. The amplifier used was an Argonaut "negative capacitance electrometer" which fed into a Tektronix type 502 dual beam oscilloscope.

Other recording techniques, the control of light to the preparation, and the making of permanent records are described elsewhere (Gwilliam, 1963). Light intensity is referred to as "unit intensity" or "intensity one" or a percentage of unit intensity achieved with neutral density filters. Unit intensity was approximately 1,000 foot-candles at the preparation.

The preparation used for external recording was achieved in the following manner: The opercular valves, bearing the body of the barnacle, were dissected free from the shell. This was then placed in a wax-lined dish, opercular plates down, and a pin thrust through the median junction of the apex of the scuta. The body was then extended along the longitudinal axis away from the terga and pinned. This exposed the mouth and ventral surface, brought the adductor muscle into view, and made dissection of the median photoreceptor, supraesophageal ganglion, and circumesophageal connectives relatively simple. In this position the lateral photoreceptors are found beneath the body of the animal close to the scutal margin just to either side of the mid-line in *B. tintinnabulum,* but would not be included in the preparation in *B. eburneus,* for in that species the eyes are displaced more basally and laterally onto the shell lining (see above).

The supraesophageal ganglion must be exposed by dissection, which then makes it possible to locate and identify the circumesophageal connectives, the antennular nerves (which contain the lateral ocellar axons) and the suprasplanchnic nerves. The area overlying the adductor muscle may be dissected away, which exposes the median ocellus and its nerve, the adductor muscle itself, and the great splanchnic nerve, with its adductor muscle motor branch. Further, one can expose the ventral ganglion and the cirral nerves to make the latter accessible for recording motor output. All this can be done without disrupting the circuit as illustrated in the diagram (Fig. 1), so that it is possible to record at any one site and remove sensory input as desired. Thus, one can record from cirral nerves or adductor muscle motor nerves with the rest of the system intact, cast a shadow, observe the effect, and then cut either the lateral or median ocellar nerve and again observe the effect of a shadow. The same procedure can be followed when recording from the circum-

esophageal connectives, but as it is necessary to cut them close to the ventral ganglion for recording, it would no longer be possible to record responses to shadows in cirral nerves or in the adductor motor supply.

Results

Structure

After dissection as described above, the terga and the scutal apex would be at the bottom of Figure 1, with the cirri extending from the top. The general body surface viewed is morphologically the ventral surface. In most cases the median ocellar nerve can be seen through the thin, usually non-pigmented exoskeleton, and the "ophthalmic ganglion" of Darwin (the median photoreceptor) can sometimes be seen lying very close to the adductor muscle, at which point it is attached. It is also usually possible to see the great splanchnic nerve which originates on the dorsal aspect of the ventral ganglion, runs out laterally to the scuta, and gives off a motor branch that supplies the adductor muscle.

The diagram in Figure 1 is based on *B. tintinnabulum*, the same species illustrated by Darwin (1854, Pl. XXVII, Fig. 2), but apart from differences in orientation of the two figures, one significant difference should be noted. Darwin assumed (but did not actually see) a connection between the lateral ocelli and what he called the ophthalmic ganglion (the median photoreceptor of Figure 1) which I cannot find. It is clear that the lateral ocellar axons enter the supraesophageal ganglion independently of the median ocellar nerve, since severing the median ocellar nerve at the supraesophageal ganglion does not interfere with responses to shadows in the rest of the system as long as the antennular nerve is intact.

In a previous paper (Gwilliam, 1963), I stated that the median photoreceptor was probably the only one present in *B. cariosus*, and that it was only occasionally functional as a photoreceptor in *B. eburneus*. I am now convinced that both of these statements are in error, for lateral photoreceptors can be demonstrated physiologically in *B. cariosus*, if care is taken not to cut too close to the scuta when dissecting the opercular plates free. The small size of *B. eburneus* and consequent difficulty in dissecting make it likely that previously the median ocellar nerve was damaged in many preparations of that species.

These new observations, and the fact that *B. tintinnabulum*, *B. balanus*, *B. crenatus* and *B. balanoides* all possess both lateral and median ocelli convinces me that all balanids probably conform to the pattern illustrated diagrammatically in Figure 1, but that the lateral ocelli are better developed and more obvious in some than in others. *B. eburneus* and *B. amphitrite* are similar in having obvious, pigmented lateral ocelli in the position described for *B. eburneus* by Fales (1928). In *B. tintinnabulum*, *B. balanus* and *B. crenatus* they are not so obvious and lie closer to the mid-line, just inside the margin of the scuta in the opercular membrane. In *B. cariosus* and *B. balanoides* the lateral ocelli have not been seen, but can be physiologically demonstrated to occupy a position similar to that in the last three species mentioned.

The structure of the photoreceptors themselves is reported by Fahrenbach (1965) for the median ocellus of *B. cariosus* and, in less detail, for the lateral ocelli of *B. amphitrite* which are virtually identical to *B. eburneus*. Fales (1928) reports two large photoreceptor cells in each lateral eye of *B. eburneus*, but there are in

fact three (based on examination of serial sections of *B. eburneus* lateral ocelli). In both the median and lateral ocelli examined, there is no ommatidial organization, and no evidence of a synaptic layer close to the ocellus. The cell bodies have finger-like "dendritic" projections which bear the microvilli, and each soma has a large axon that apparently does not synapse until the level of the supraesophageal ganglion. The size of the axons (15–20 μ in diameter) and the nature of the glial

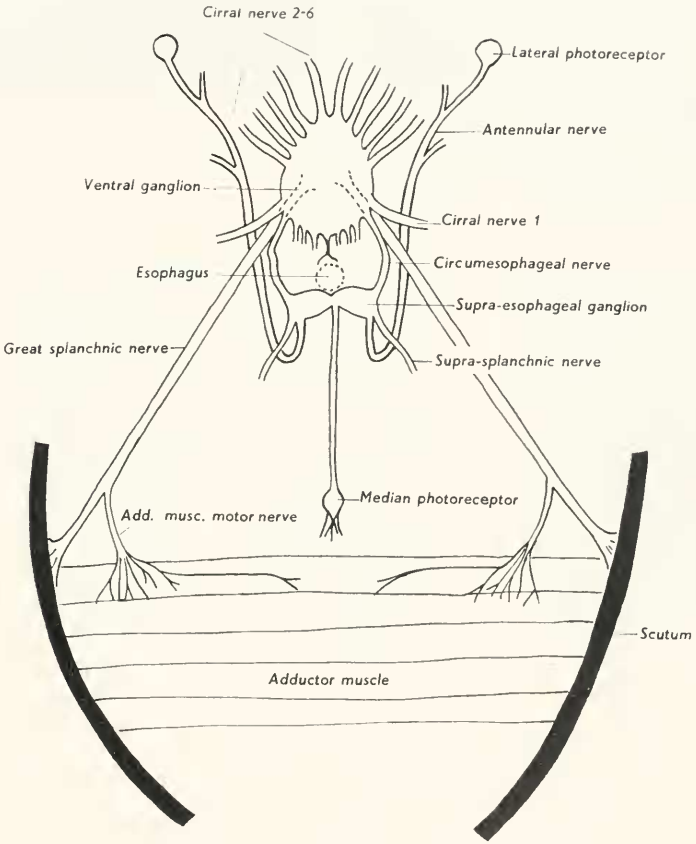

FIGURE 1. Diagram of the balanid central nervous system, showing the relationship of the photoreceptors to it. Based on *B. tintinnabulum*. Details of branching in the antennular nerve are schematic and are included simply to indicate that the nerve is mixed.

sheath around the ocellar nerve suggest a high value for the length constant of the axons.

Electrical activity

(a) *Balanus eburneus:* intracellularly recorded responses

Although direct proof of penetration of photoreceptor cells is lacking, it was assumed when a maintained negative potential was recorded. Further, only those preparations which showed reversible depolarization when exposed to a light flash

were assumed to have been successfully impaled. Such cells could often be held for as long as three hours, but relatively few such preparations were obtained. In the limited time available, only a total of twelve preparations met the above criteria for any significant length of time.

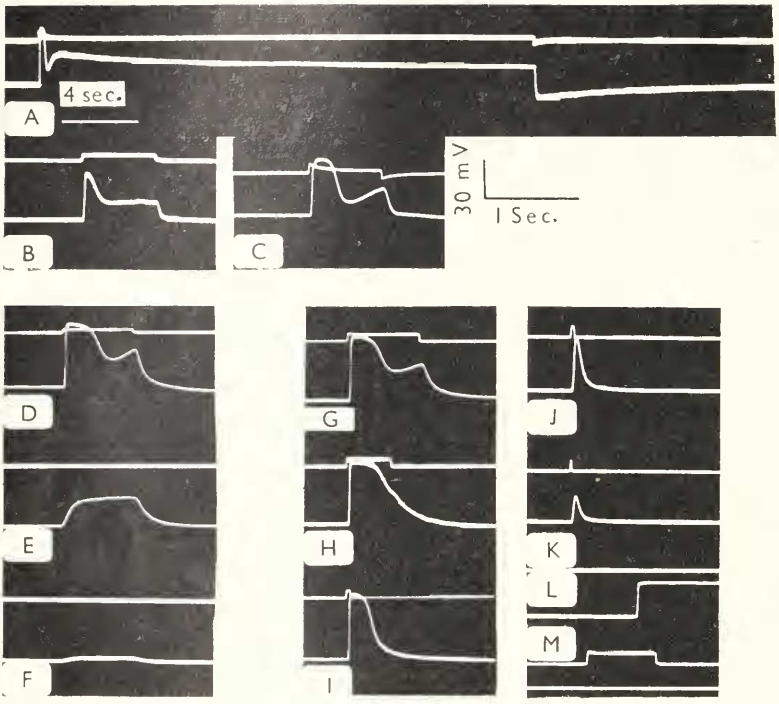

FIGURE 2. Intracellular records from the lateral photoreceptor cells of *Balanus cburneus*. Inset time calibration applies to B through M; voltage calibration applies to all records. In this figure and all others, upward deflection of the second beam indicates "on." A, sustained response. B and C, the response to a 0.8-sec. light flash, B at a lower intensity than C. D, response to a flash of unit intensity. E, 1.0% unit intensity. F, 0.1% unit intensity. Photocell failed to record in E and F. G–K, Decreasing time series. L, The membrane potential at the close of the time series. M, the effect of a light flash on the removed electrode.

In such cells, membrane potentials recorded varied considerably, depending on the immediate history of the penetrated cells. Initial membrane potentials recorded on penetration while viewing in relatively bright light were on the order of 30–45 mV, inside negative. After one hour in darkness these approached 60–70 mV.

The wave-forms of the potentials recorded when the preparation was exposed to a flash of light are shown in Figure 2. This consists of the familiar "on" transient, often, but not always, a secondary rise, followed by a maintained level of depolarization. At "off" this drops very close to the original membrane potential level (Fig. 2, A, B, C). Amplitude of the generator potential was graded in different light intensities (Figure 2, D, E, F), and the transient disappeared at low intensities. The wave-form also varied in light flashes of intensity one, but of

different duration (Fig. 2, G–K). In this case only the transient remained in flashes less than 0.5 second in duration.

At the highest intensity the transient may overshoot zero potential, but this could not be determined with certainty, because of the shifting membrane potential dependent on previous exposure to light (*cf.* Naka, 1961; Naka and Eguchi, 1962a) and to the D. C. drift in the amplifier. However, in a dark-adapted preparation, the transient seldom exceeded 55 mV, which suggests that overshoot did not occur if membrane potentials reached the values of 60–70 mV which were recorded in other cells after dark adaptation.

It will be noted that these intracellular responses are very similar in form to the presumed intracellular response from the median eye of *B. cariosus* as previously reported (Gwilliam, 1963, p. 476) and very similar to the simple electroretinograms recorded from barnacle ocellar nerves, if the difference between A. C. and D. C. recording is taken into account. That is, the extracellularly recorded "mass" response is directly comparable to the single unit intracellularly recorded response, both being almost certainly uncomplicated by post-synaptic events.

Under the conditions of the observations reported here, it seems highly unlikely that the "on" transient has its source in other than the impaled retinula cell. The photoreceptor consists of three primary receptor cells, supporting cells, and very little else. There are no nearby post-synaptic cells to contribute, so the suggestion that the "on" transient originates elsewhere (Burkhardt and Autrum, 1960; Burkhardt, 1962) seems to be ruled out in this material. As Ruck (1964) points out, the recorded amplitude alone of the transient argues very strongly against its origin outside the retinula cell.

The records are also uncomplicated by anything resembling ordinary spikes. This is also true of the ocellar-nerve recorded ERG when the bundle is uncontaminated with other nerve fibers. There is thus no evidence that the retinula cell axons conduct ordinary spikes, despite the relatively great distances over which they presumably transmit.

It may be argued that in the illustrated cases the photoreceptor cell axons have been damaged in the exposure procedure, and that this could in turn destroy the spiking locus. However, if the "on" transient is accepted as an axonal event, its presence in these records argues against extensive axonal damage. It might also be argued that the light levels used are insufficient to operate the spiking mechanism, but the same light levels serve to inhibit firing of cells in the supraesophageal ganglion, proving that they are adequate to operate the normal post-synaptic inhibitory mechanism that leads to the shadow reflex upon release.

The suggestion put forth by Ruck (1964) that the transient may be a regenerative event has not been adequately tested in this material, but as Ruck himself points out, this will not account for sustained transmitter action on post-synaptic cells.

(b) External recording in *B. tintinnabulum* and *B. cariosus*

1. Lateral *vs.* median photoreceptor function

Having established that two morphologically distinct sets of photoreceptors existed, I tried to discover if they had different functions. The records reproduced in Figure 3 illustrate the results of observations on the two species. Figure 3, A

illustrates a circumesophageal connective recording of the results of a shadow cast on a preparation of *B. cariosus* with both sets of photoreceptors intact. Figure 3, B was taken from the same preparation after severing the median ocellar nerve, and Figure 3, C after severing both antennular nerves. Figure 3, D is from a different preparation of the same species, the electrode recording from the motor

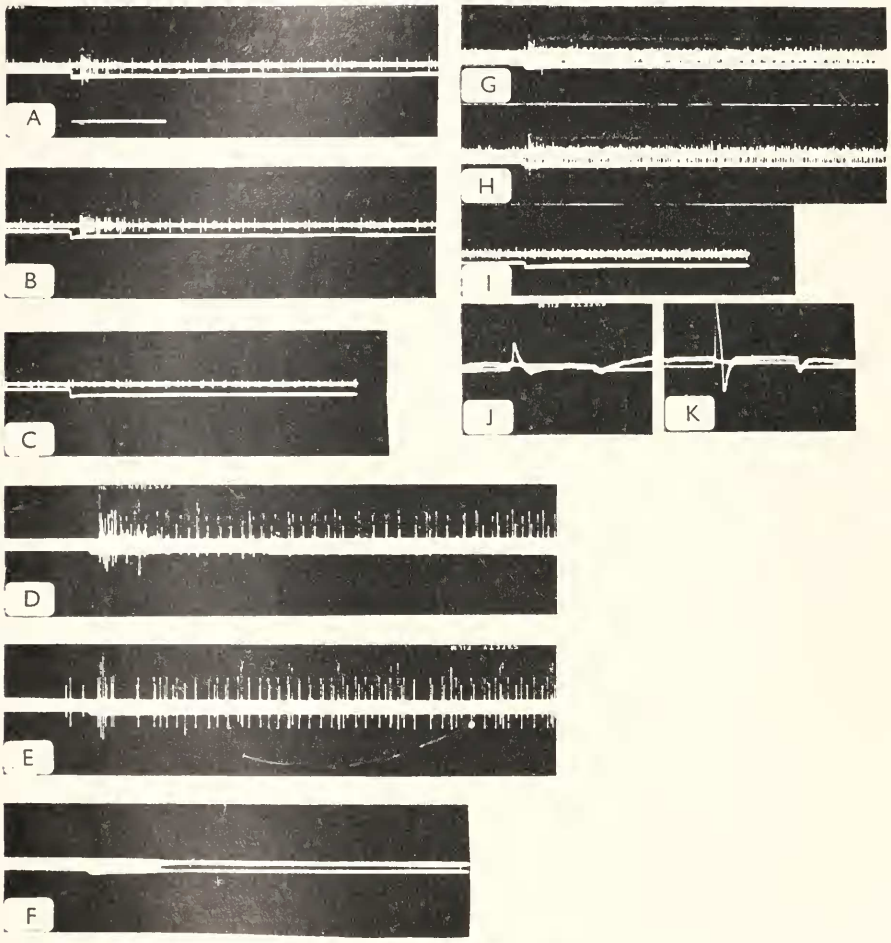

FIGURE 3. Function of the lateral and median eyes in *B. cariosus* (A–F) and *B. tintinnabulum* (G–K). See text for explanation.

supply to the adductor muscle, with both sets of photoreceptors intact. Figure 3, E is from the same preparation with the antennular nerves severed, and in Figure 3, F the median nerve has been severed as well.

These records prove the existence of lateral photoreceptors in *B. cariosus,* establish that both sets are capable of mediating the shadow reflex, and suggest that there is no difference in function between the lateral and median photoreceptors in this particular pathway.

Figure 3, G is a circumesophageal recording from *B. tintinnabulum* with both sets of eyes intact; in H, the antennular nerves have been cut; and in I, the median nerve was also severed. Recordings from the adductor motor supply give the same results as in *B. cariosus*. These records, also, indicate that there is no difference in function of the two sets of photoreceptors in *B. tintinnabulum*.

Figure 3, J and K were obtained from the cut ends of the nerves containing the photoreceptor axons. J is a record of the lateral ocellar ERG taken from the cut end of the nerve close to the supraesophageal ganglion, while K was recorded from the same relative position from the median nerve. Both records were taken at the same overall gain and band pass frequency (0.3–2000 cps) and at the same dis-

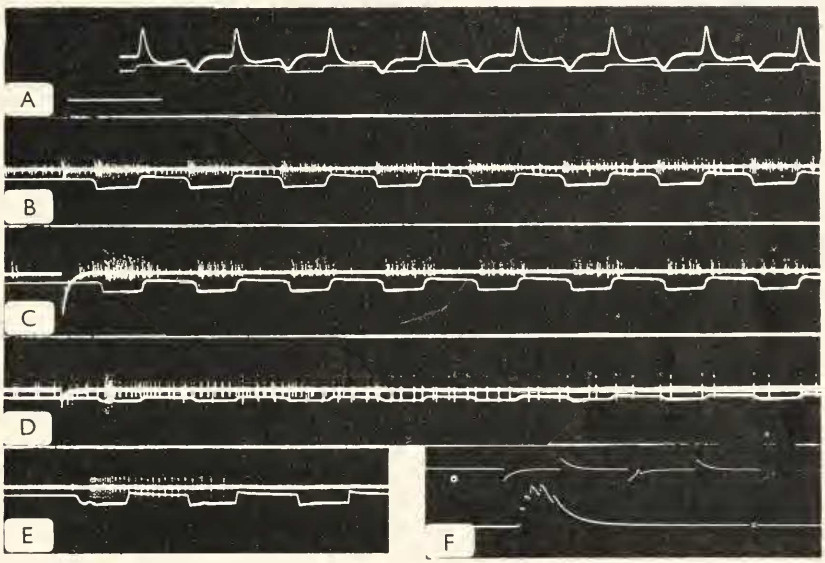

FIGURE 4. Series to illustrate different rates of adaptation to multiple stimuli at different points in the responding system. Upward deflection of upper beam in A and lower beam in F indicates *positivity* of active electrode. Membrane potential in F (indicated by the initial separation of the two beams) = 60 mV. In F, downward deflection of upper beam indicates "off."

tance above the bathing medium. The difference in amplitude, rise and decay times in the two records may reflect the greater distance of transmission of the lateral ocellar axons (Fig. 3, J) and probably illustrates the decremental nature of ocellar axon transmission.

It should be pointed out at this juncture that the initial deflection of the ERG when recording externally from the ocellar nerve at some distance from the photoreceptor with a single active electrode is positive in sign rather than negative as erroneously reported in Gwilliam (1963). If the record is taken just distal to, or from the region of the photoreceptor cells, the sign is reversed. This result then accords with other arthropod ocelli in which the ERG is cornea-negative and retinula cell axon-positive when recorded extracellularly, as shown by Ruck (1961) and others.

2. The response to multiple shadows

Gwilliam (1963) briefly reported that the response to multiple shadows at different points in the photoreceptor-motor output chain showed different rates of adaptation. This was investigated more fully in *B. tintinnabulum* and *B. cariosus*, two species obtained from rather different habitats and showing different behavioral reactions to multiple shadows. The similarities and differences between the two species are illustrated in Figure 4.

Figure 4, A illustrates the non-adapting nature of the ERG in *B. cariosus*, and identical records have been obtained from *B. tintinnabulum*. Figure 4, B is a record of a circumesophageal recording from *B. tintinnabulum* which illustrates that at this point (the presumed second-order neurons) adaptation is very slow, but will fail to follow after approximately 30 shadows of the duration and frequency shown. A very similar phenomenon can be demonstrated in the circumesophageal connective of *B. cariosus*. Similarly, the motor output to the cirri in *B. tintinnabulum* adapts very slowly (Fig. 4, C), but the cirral output in *B. cariosus* adapts very rapidly, often failing to follow even after a single shadow (Fig. 4, D), and seldom persisting for more than four shadows. In both species the motor output to the adductor muscle fails to follow after 1–4 shadows (Fig. 4, E of *B. cariosus*). Figure 4, F is a record of an intracellular response of one of the giant muscle fibers from the adductor muscle in *B. cariosus* and illustrates the effect of a burst of motor nerve action potentials on the muscle junctional potentials (*cf.* Fig. 4, E) in response to a shadow.

If one now turns to an intact, feeding animal and presents shadows of the same duration and frequency used in the neurophysiological work, the behavior of each species corresponds to the pattern seen in the records of Figure 4.

B. tintinnabulum will respond by withdrawal of the cirri and valve closure (adductor muscle contraction) to the first shadow. If shadow-casting is continued, the animal very quickly emerges, but continues to withdraw the cirri at each shadow, but after one or a few additional shadows fails to close the valves.

B. cariosus, on the other hand, responds to the first shadow, quickly re-emerges and, after one to four additional shadows, proceeds to execute "fishing" activities, completely ignoring the changing light level.

These responses, of course, will occur in this particular way only in the absence of any reinforcing stimuli such as mechanical shocks, or tactile stimuli. If the shadow is accompanied by a tactile stimulus or a blow to the dish containing the animals, they remain closed for much longer periods of time and do not adapt to the dual stimulus nearly so quickly.

While this difference in behavior is difficult to explain with any degree of confidence, it is interesting to note that the *B. cariosus* used in this study were collected from the outer Oregon coast where the wave action may be severe, and the water frequently contains much floating and suspended debris. *B. tintinnabulum*, however, was collected from harbor floats and pilings in relatively quiet bays in southern California. In the two differing situations, it may be that a shadow is a more "urgent" stimulus in quiet water (*i.e.*, more frequently signals the approach of a predator), and continued response is of significant value to the species. In more turbulent waters, where shadows quite often signal only a piece of floating debris, the response may be less significant.

Discussion

The information now available on the structure and function of the adult barnacle photoreceptors and the nervous system permits a résumé which represents a fairly complete description of, at least, the obvious pathways and events that are involved in the shadow reflex. In no case has the response chain, from photoreceptor cell membrane depolarization to muscle junctional potentials, been followed completely through in one species, but by combining information from several it is possible to reconstruct the probable chain of events.

It now seems highly probable that all balanid cirripedes possess two distinct sets of photoreceptors: a pair of bilaterally symmetrical lateral ocelli and a single median photoreceptor "ganglion." That these receptors contain retinula cells with typical arthropod rhabdomere microvilli is now established, and the absence of a synaptic layer close to the retinula cells is strongly indicated (Fahrenbach, 1965).

It is generally held that the adult cirripede eye(s) takes its origin from the median eye of the nauplius larva (*e.g.*, Doochin, 1951), and the structure of that eye in the larva of *Balanus* suggests the developmental source of the three separated photoreceptors found in the adult. While a detailed comparison of the structure of adult and larval eyes (Kauri, 1962; Fahrenbach, 1965) reveals considerable difference in numbers of sensory cells and their organization, the existence of three components in the larva is very suggestive. It should be recognized that many larval structures do not develop directly into adult structures but emerge as the definitive adult structures following a phase of larval "degeneration" (Bernard and Lane, 1962).

To judge from the location and structure of the two sets of "eyes" in an animal like *B. cburneus,* it would seem that the median photoreceptor receives light most easily when the animal has the opercular valves open and the cirri extended. However, it must be recognized that an actively "fishing" barnacle probably casts shadows on its own median photoreceptor, which suggests the existence of inhibitory feed-back mechanism to prevent withdrawal reactions during this process.

In the lateral photoreceptors of *B. cburneus,* the location of light-absorbing and reflecting pigments over the inner surface of the PR cells makes it apparent that they must receive light either parallel to the shell plates and/or from the outside. In an animal like *B. tintinnabulum* this may also be true, but the inner shielding is less well developed. Very little can be said about *B. cariosus* lateral eyes, for the structure has not yet been morphologically identified.

The structural information on the retinula cells provided by Fahrenbach (*loc. cit.*) helps a great deal in explaining the absence of propagated action potentials in barnacle retinula cell axons. The large size of these axons (15–20 μ diameter) contrasts markedly with those of, for example, the cockroach dorsal ocellus which averages 0.5 μ (Ruck, 1964). Also, the inter-axonal space which is filled with glial cell membrane is quite large, so that in contrast to the cockroach, the length constant of barnacle retinula cell axons is probably quite large. It thus appears that a structural basis for long-distance electrotonic conduction is present.

The function of these photoreceptors seems to be primarily that of initiating the shadow reflex, although other functions may also be imagined. Structural considerations rule out any image-forming capabilities, and it seems evident that the "eyes" are relatively simple light-level and transient-photic-event monitors. No

difference in function of the lateral and median photoreceptors is so far apparent with the techniques used in this study, but more subtle functional differences are not precluded.

In a purely speculative manner, one might imagine the sequence of events leading to a shadow reflex as occurring in the following way: self-cast shadows on the median photoreceptor would cause a certain amount of depolarization in the second order neurons at "off." In an immobilized preparation this would be sufficient to trigger the reflex, but in a "fishing" animal this would be countered by inhibitory neurons (acting on the same second-order cells) activated by the body movements. The balance between these two processes and the inhibitory influence of the illuminated lateral photoreceptors would serve to keep the second-order cell membrane potential depressed below the firing level. If, during this process, the added depolarization (release from inhibition) furnished at "off" by the lateral photoreceptors should impinge on the second-order neurons, the firing level would be reached, which would operate the withdrawal-closure reflex; this would in turn shut off the inhibitory feed-back mechanism and keep the animal contracted until the shadow was removed, or until firing in the second-order cells ceased (a matter of approximately 30–60 seconds).

That there are distortion-sensitive sensory cells present in the mantle lining close to the body can be demonstrated by stretching the mantle while recording from the cut end of the antennular nerve, the same nerve that carries the lateral photoreceptor axons. The spikes shown in Figure 10 (page 482) of the previous paper (Gwilliam, 1963) are almost certainly in this category. Whether or not they inhibit the same second-order cells or the motor cells involved in the shadow reflex is not known. Hoyle and Smythe (1963) have been unable to demonstrate peripheral inhibition in barnacles, but central inhibition certainly occurs.

However, the chain of events as demonstrated to date suggests the following interpretation:

(a) The photoreceptor cells generate a sustained depolarizing potential when illuminated.

(b) This potential is transmitted by passive electrotonic conduction *via* the large retinula cell axons to the supraesophageal ganglion where the axons synapse with the second-order neurons.

(c) The sustained depolarization probably causes the continual release of an inhibitory transmitter substance from the terminations of the retinula cell axons which prevents the second-order cells from firing, although inhibition may be accomplished by some other mechanism.

(d) At "off," the inhibition is released and the second-order cells begin to fire.

(e) The second-order cells synapse either directly or through other interneurons in the ventral ganglion with motor neurons. At this level the post-synaptic event is excitatory.

(f) At this level, rather than the previous one, the phenomenon of synaptic failure (seen as a failure of motor neurons to respond to multiple stimuli) probably occurs. This "tendency to failure" varies in different species and in different motor cells of the same species.

It thus appears that the barnacle retinula cell behaves in a very similar fashion to the retinula cell in the insect dorsal ocellus (Ruck, 1962, for summary), the

striking difference being the greater distance between the retinula cell and the first synapse. This has apparently been compensated for in the barnacle by the morphological specializations referred to previously rather than by the development of an impulse-propagating mechanism. It seems quite plausible to argue that this mode of transmission is fundamental to arthropod photoreceptor cells, and the existence of a spiking mechanism (Naka and Eguchi, 1962b) represents a high degree of specialization. Washizu (1964), recording intracellular potentials from blowfly compound eyes, detected no impulse activity and demonstrated that the "on" transient did not overshoot zero potential and was graded. Unequivocal evidence of propagated impulse activity in retinula cell axons, on the other hand, is very limited.

Summary

1. The gross structure of the balanid central nervous system and some of the peripheral structures involved in the shadow reflex are described and figured (Fig. 1). The existence of both paired lateral and single median photoreceptors in several species of barnacles is established, and is probably true for all balanid cirripedes.

2. Intracellular sensory potentials from the lateral ocelli of *B. eburneus* indicate that spiking does not occur in these retinula cells, and that the wave form of the response to a light flash is very similar to comparable records from other arthropod retinula cells.

3. No significant difference between the function of the lateral and the median ocelli has been shown with the procedures used in this study.

4. The different rates of adaptation of neurons in the reacting chain have been studied. The primary sensory event is non-adapting, the presumed second-order neurons adapt very slowly, as does the cirral motor output in *B. tintinnabulum*. The cirral output in *B. cariosus*, however, adapts rapidly, and so does the adductor muscle motor output in both species. This difference in motor output correlates very well with the behavior of intact animals.

5. The probable chain of events leading to the withdrawal-closure response to a shadow is summarized.

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